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6. AUTHOR(S)
Riley, Margaret A.
Roy, Sandra M.

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Massachusetts Amherst
70 Butterfield Terrace
Amherst, MA 01003-9242

8. PERFORMING ORGANIZATION REPORT NUMBER
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9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Office of Naval Research
Boston Regional Office
495 Summer Street, Suite 627
Boston, MA 02210-2109

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14. ABSTRACT
Bacteriocins active against clinically-relevant Pseudomonas aeruginosa, Staphylococcus aureus, Acinetobacter baumanii, and Bacillus cereus have been identified and their killing activity, stability, and protein sequences have been assessed.

15. SUBJECT TERMS
Bacteriocins, Antimicrobials, Pseudomonas aeruginosa, Staphylococcus aureus, Acinetobacter baumanii, Bacillus cereus

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Year One

Phase one is to identify a minimum of 3 bacteriocins active against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Acinetobacter baumanni* and *Bacillus cereus* (target pathogens for the proposed research).

Summary:
We have identified at least 3 putative bacteriocins for all target pathogens. It should be noted that the bacteriocin candidates active against Bacillus are active against the genus in general and not specifically against *Bacillus cereus* (*B. cereus* isolates were not included with the Bacillus strains obtained from Natick for screening). Also, the candidates active against *S. aureus* are produced by *P. aeruginosa*. Table 1 shows the inhibitory activity of several candidate bacteriocins against the target pathogens (recorded as number of strains inhibited per total target screened).

Table 1. List of putative bacteriocins active against targets

Candidate	Target	Activity
FD8x	Bacillus	10/17
FD10f	Bacillus	7/17
FD8u	Bacillus	7/17
PA-15	*S. aureus*	14/20
PA-18	*S. aureus*	14/20
PA-31	*S. aureus*	12/20
PA-15	*P. aeruginosa*	28/36
PA-18	*P. aeruginosa*	29/36
PAF41	*P. aeruginosa*	29/36
A51	*A. baumanii*	10/10
A64	*A. baumanii*	10/10
A65	*A. baumanii*	10/10

Phase two involves assaying the killing breadth of each bacteriocin against a panel of target pathogens and a panel of strains representing the diversity of bacteria in the “Warfighters local environment” (commensals).

Summary: Our collaborators at NSRDEC asked us to focus on the *P. aeruginosa* and *S. aureus* bacteriocin candidates, but we also included the Bacillus candidates as well. We screened all candidates against a large panel of 10 species of uropathogens (minimum of 19 strains/species) to assess their killing breadth. This was done using crude lysates of the bacteriocin candidates both using a 96-pin replicator (~1ul of lysate) and a repeat-pipettor (5 ul of lysate). The results of this screen can be seen in Tables 2A and 2B respectively. This data was provided to

NSRDEC. The commensal screen was put on hold as commensal organisms were not available from NSRDEC and the pathogen screen was deemed much more relevant and informative.

Table 2A. Uropathogen Screen Results Using 96-pin replicator (1ul lysate)

	Pyocin/ Pyocin-like															
	PML 28	NIH 18	PAF 41	PYS 3.3	PA01	PA-14UCCP	CD-14	CD-24	CD-68	CD-81	CD-91	PA-15	PA-18	PA-25	PA-31	PA-36
Total E. coli killed (100)	0	0	0	0	0	0	2	2	0	0	0	2	3	7	3	1
% of E. coli killed	0%	0%	0%	0%	0%	0%	2%	2%	0%	0%	0%	2%	3%	7%	3%	1%
Total Pseudomonas killed (34)	25	18	27	4	17	20	28	23	20	12	24	27	28	14	12	15
% of Pseudomonas killed	74%	53%	79%	12%	50%	59%	82%	68%	59%	35%	71%	79%	82%	41%	35%	44%
Total Acinetobacter killed (3)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
% of Acinetobacter killed	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
Total Candida killed (1)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
% of Candida killed	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
Total Klebsiella killed (20)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
% of Klebsiella killed	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
Total Proteus killed (20)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
% of Proteus killed	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
Total Enterococcus killed (20)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
% of Enterococcus killed	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
Total Staphylococcus killed (20)	0	2	8	0	9	13	13	1	6	13	3	14	14	0	12	12
% of Staphylococcus killed	0%	10%	40%	0%	45%	65%	65%	5%	30%	65%	15%	70%	70%	0%	60%	60%
Total Citrobacter killed (20)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
% of Citrobacter killed	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
Total Enterobacter killed (20)	0	0	0	0	0	0	1	1	0	0	0	1	1	0	0	0
% of Enterobacter killed	0%	0%	0%	0%	0%	0%	5%	5%	0%	0%	0%	5%	5%	0%	0%	0%
Total Strains Killed (259)	25	20	35	4	26	33	44	27	26	25	27	44	46	21	27	28
Total % of Strains Killed	10%	8%	14%	2%	10%	13%	17%	10%	10%	10%	10%	17%	18%	8%	10%	11%

Table 2B. Uropathogen Screen Results Using Multi-channel Pipette (5ul lysate)

	Pyocin/ Pyocin-like															Bacillus			
	PML28	NIH18	PAF41	PA01	PA-14UCCP	CD-14	CD-24	CD-68	CD-81	CD-91	PA-15	PA-18	PA-25	PA-31	PA-36	FD6ee	FD8u	FD8x	FD10f
Total E. coli killed (100)	1	1	0	0	0	2	2	0	0	2	0	1	0	1	0	0	1	1	1
% of E. coli killed	1%	1%	0%	0%	0%	2%	2%	0%	0%	2%	0%	1%	0%	1%	0%	0%	1%	1%	1%
Total Pseudomonas killed (40)	26	25	36	20	22	34	29	29	11	30	7	30	2	14	19	0	0	0	0
% of Pseudomonas killed	65%	63%	90%	50%	55%	85%	73%	73%	28%	75%	18%	75%	5%	35%	48%	0%	0%	0%	0%
Total Klebsiella killed (40)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
% of Klebsiella killed	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
Total Acinetobacter killed (19)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
% of Acinetobacter killed	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
Total Proteus killed (41)	0	0	0	0	1	2	0	1	1	0	0	1	0	1	1	0	0	0	0
% of Proteus killed	0%	0%	0%	0%	2%	5%	0%	2%	2%	0%	0%	2%	0%	2%	2%	0%	0%	0%	0%
Total Bacillus killed (20)	0	0	0	1	9	10	0	2	10	0	0	11	0	10	9	0	4	11	4
% of Bacillus killed	0%	0%	0%	5%	45%	50%	0%	10%	50%	0%	0%	55%	0%	50%	45%	0%	20%	55%	20%
Total Enterococcus killed (40)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0
% of Enterococcus killed	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	13%	0%
Total Staphylococcus killed (40)	1	13	4	29	33	36	3	23	34	8	11	39	3	35	35	0	1	2	0
% of Staphylococcus killed	3%	33%	10%	73%	83%	90%	8%	58%	85%	20%	28%	98%	8%	88%	88%	0%	3%	5%	0%
Total Citrobacter killed (23)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
% of Citrobacter killed	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
Total Enterobacter killed (33)	0	0	1	0	0	0	1	1	0	0	0	1	0	0	0	0	0	0	0
% of Enterobacter killed	0%	0%	3%	0%	0%	0%	3%	3%	0%	0%	0%	3%	0%	0%	0%	0%	0%	0%	0%
Total Strains Killed (259)	**28**	**39**	**41**	**50**	**65**	**84**	**35**	**56**	**56**	**40**	**18**	**83**	**5**	**61**	**64**	**0**	**6**	**19**	**5**
Total % of Strains Killed	**7%**	**10%**	**10%**	**13%**	**16%**	**21%**	**9%**	**14%**	**14%**	**10%**	**5%**	**21%**	**1%**	**15%**	**16%**	**0%**	**2%**	**5%**	**1%**

Year Two

Phase three involves assaying bacteriocin activity in different environmental conditions.

Summary: Candidates were assayed under 37 different environmental conditions including temperature, salinity, acidity, salinity, and other chemicals. The pyocins and bacillus bacteriocins tended to lose activity under most conditions. Our collaborators at NSRDEC are attempting to purify these proteins to resolve this stability issue. Details of the experiments are provided below.

Bacteriocin Stability Study

Goal. The focus of this study is to assess the stability of a suite of bacteriocins that are of interest to our Army Natick Lab collaborators. They are interested in testing the effect of time, temperature, chemicals, detergents, and enzymes on the efficacy of their bacteriocins. These bacteriocins were identified from our strain collection as possessing activity against a panel of strains of interest to the army. The most effective killers were chosen for further research – as listed in **Table 1** below.

Table 1. Bacteriocins of Interest to the Army

Bacteriocin	Species	Lawn
PA-15 – lysate	*P. aeruginosa*	NIH3
PA-18 - lysate	*P. aeruginosa*	NIH3
PA-31- lysate	*P. aeruginosa*	NIH3
PA-41- lysate	*P. aeruginosa*	NIH3
FD7g- lysate	**Bacillus spp*	FD10c
FD8u- lysate	**Bacillus spp*	FD10c
FD8x- lysate	**Bacillus spp*	FD10c
FD10f- lysate	**Bacillus spp*	FD10c
ColE1 - lysate	*E. coli*	BZB1011
ColE1 - pure	*E. coli*	BZB1011

Bacteriocin Production Methods. Unless otherwise noted – all bacteriocins were grown as follows: overnight growth in LB, at 37C, 250rpm. Transfer 10 ul into 10 ml fresh LB, grow until ~$1x10^7$ cells/ml. Add mitomycin C at final concentration of 150ug/ml. Grow for 2.5 hours. Add 0.5 ml chloroform. Vortex. Spin at 10K rpm, 4 C for 10 min. Transfer supernatant into fresh tubes and store at 4C or -20C. **For Bacillus strains, no MitC and no chloroform are added and the cells are grown in BHI at 30C and 150 rpm.

Pure Bacteriocins. Pure colicin E1 was obtained from Sigma (250 ug/ml). Pure FD7g was obtained from Army (250 ug/ml). Both stored at -20C.

Time and Temperature Bacteriocin Stability Study.

Goal. The goal of this study was to assess bacteriocin stability over the course of time at 5 temperatures.

Aim 1. Make fresh lysates and test for killing.

Methods. Fresh lysates made and stored at 4C. Each lysate assayed on lawn. Pure colicin E1 was obtained from Sigma (250 ug/ml). Pure FD7g was obtained from Army (250 ug/ml).

Table 2. Lysate Killing Assay

	Bacteriocin	Lawn	Assay
1	PA-15 – lysate	NIH3	0.5
2	PA-18 - lysate	NIH3	0.5
3	PA-31- lysate	NIH3	0.5
4	PA-41- lysate	NIH3	1
5	FD7g- lysate	FD10c	1
6	FD7g - pure	FD10c	0
7	FD8u- lysate	FD10c	0.25
8	FD8x- lysate	FD10c	0.25
9	FD10f- lysate	FD10c	0.25
10	ColE1 - lysate	BZB1011	1
11	ColE1 - pure	BZB1011	1

Results. Table 2 provides results from lysate killing assays (1 = killing zone, 0.5 hazy, weak killing zone, 0 = no killing zone). The pseudomonas lysates (PA-15 – AP41) all killed, although 15, 18, and 31 produced weak, hazy zones. These lysates were remade and the same weak zone resulted. The bacillus lysates produce very hazy zones (FD7g – FD10f). The FD7g pure bacteriocin did not produce a killing zone. The bacillus lysates were remade and the same zone resulted. The E. coli lysate (ColE1) and pure colicin (ColE1 - pure) produce large clear zones.

Conclusions. Prior studies had also shown that the bacillus and pseudomonas lysates tended to show very hazy killing zones. It was determined that we would proceed with the pseudomonas lysates and FD7 lysate. Discussions with the Army resulted in our NOT proceeding with the pure FD7g – they are going to work on getting more protein.

Aim 2. Set up study to test the impact of the following temperatures, times, and chemicals on killing efficacy.

Methods – Time, temperature, and chemical exposure. Using the lysates described above, small eppendorfs received 30 ul of lysate and were then stored at the prescribed temperature and sampled at the prescribed times. Three replicates were spotted onto appropriate sensitive lawns and measured to the nearest 0.1 mm. Pure colicin E1 was thawed from -20 and plated to assay the efficacy after repeated freeze/thaws. Lysates were also tested for activity after exposure to a panel of chemicals, temperatures, and freeze/thaw **(Table 4)**.

Time & Temperature Results (Tables 3A – 3F).

- Pyocin PA-15 lysate lost activity at 65C immediately (after 6 hours) and then lost activity at 25 C and 37 C within 1 day, and then lost activity at 4 C by 2 days **(Table 3A)**.

- Pyocin PA-18 lysate lost activity at 65C immediately (after 6 hours) and then lost activity at 25 C and 37 C within 1 day, and then lost activity at 4 C by 5 days. **(Table 3B)**.
- Pyocin PA-31 lysate lost activity at 65C immediately (after 6 hours) and then lost activity at 37 C within 1 day, at 25 by 2 days and then lost activity at 4 C by 5 days. **(Table 3C)**.
- Pyocin AP41 lysate lost activity at 65C immediately (after 6 hours) and then lost activity at 37 C and 25C by 5 days. **(Table 3D)**.
- Bacillus bacteriocin FD7 lysate lost activity at 65C immediately (after 6 hours) and then lost activity at 37 and 25C by 1 day. It retained activity at 4 C and -20C for over 5 days. **(Table 3E)**.
- Colicin E1 lysate lost activity at 65C immediately (after 6 hours) and retained activity at all other temperatures over the course of 5 days. **(Table 3F)**.
- Colicin E1 pure retained full activity after 5 freeze thaws. **(Table 3F)**.

Chemical Exposure Results (Table 4).

- No information could be gleaned from the tests with chloroform, bleach, sodium hydroxide, or 0.5M EDTA due to the intrinsic antimicrobial nature of the chemical; seen in yellow.
- Colicin E1 lysate was inactivated by Palmolive, Softsoap, proteinase K, trypsin, and autoclaving.
- Pure colicin E1 was inactivated by Palmolive, proteinase K, trypsin, boiling, autoclaving, and microwaving.
- Nisin was not effected by any of the chemicals it was exposed to.
- Pyocin AP41 was inactivated by Palmolive, Softsoap, proteinase K, trypsin, boiling, and autoclaving.
- Bacillus bacteriocin FD7g was inactivated by apple juice, sucrose, proteinase K, trypsin, and autoclaving.

Conclusions. It is probably not worth pursuing several of the bacteriocins, due to their limited stability. Pyocins 15, 18, and 31 routinely had hazy weak zones and lost most of their activity at most temperatures within a few days. This result is consistent with prior studies by Morgan Dennis, who tried for over one year to produce better pyocin lysates and he lost activity in almost every case, even when stored at -20C. The Bacillus bacteriocins are also fairly weak. They are hard to produce lysates and the resulting lysates are weak at best and FD7g lost activity at 65, 37, and 25C within 2 days. In sharp contrast, the colicin E1 lysate and the pure colicin E1 performed well in all temperatures save 65 C.

Future Work. I will propose to the Army that we pursue nisin and several colicins to further assess their stability under a wider variety of conditions, and on different types of materials. I think it is also worth pursuing AP41, as it does produce reasonable zones and had better activity than any other pyocin in our sample.

Table 3A. Pyocin PA15 Stability Data

Pyocin PA15 Stability Data								
		trmt	rep1	rep2	rep3	mean	sd	pheno
t = 0 hr	control	4.00	1.25	1.00	0.80	1.02	0.23	med
t = 6 hr	Pyocin PA15	-20.00	1.2	0.9	1.0	1.0	0.2	weak
		4.00	1.3	1.1	1.1	1.2	0.1	weak
		25.00	1.1	0.9	1.0	1.0	0.1	weak
		37.00	1.0	1.0	0.7	0.9	0.2	weak
		65.00	0.0	0.0	0.0	0.0	0.0	none
	control	4.00	1.1	1.0	0.9	1.0	0.1	weak
t = 1 day	Pyocin PA15	-20.00	1.0	1.0	0.9	1.0	0.1	weak
		4.00	0.9	0.9	0.8	0.9	0.1	weak
		25.00	0.0	0.0	0.0	0.0	0.0	none
		37.00	0.0	0.0	0.0	0.0	0.0	none
		65.00	0.0	0.0	0.0	0.0	0.0	none
	control	4.00	1.0	1.0	1.0	1.0	0.0	weak
t = 2 day	Pyocin PA15	-20.00	1.0	0.9	1.0	1.0	0.1	weak
		4.00	0.0	0.0	0.0	0.0	0.0	none
		25.00	0.0	0.0	0.0	0.0	0.0	none
		37.00	0.0	0.0	0.0	0.0	0.0	none
		65.00	0.0	0.0	0.0	0.0	0.0	none
	control	4.00	0.0	0.0	0.0	0.0	0.0	none
t = 5 days	Pyocin PA15	-20.00	1.0	0.8	1.0	0.9	0.1	weak
		4.00	0.0	0.0	0.0	0.0	0.0	none
		25.00	0.0	0.0	0.0	0.0	0.0	none
		37.00	0.0	0.0	0.0	0.0	0.0	none
		65.00	0.0	0.0	0.0	0.0	0.0	none
	control	4.00	0.0	0.0	0.0	0.0	0.0	none
t = 10 days	Pyocin PA15	-20.00						
		4.00						
		25.00						
		37.00						
		65.00						
	control	4.00						

Table 3B. Pyocin PA18 Stability Data

Pyocin PA18 Stability Data								
		trmt	rep1	rep2	rep3	mean	sd	pheno
t = 0 hr	control	4.00	1.0	0.8	1.2	1.0	0.2	med
t = 6 hr	Pyocin PA18	-20.00	1.2	0.8	1.0	1.0	0.2	weak
		4.00	1.0	0.9	1.0	1.0	0.1	med
		25.00	0.9	1.1	1.0	1.0	0.1	weak
		37.00	1.1	0.9	1.0	1.0	0.1	weak
		65.00	0.0	0.0	0.0	0.0	0.0	none
	control	4.00	1.1	1.0	0.9	1.0	0.1	weak
t = 1 day	Pyocin PA18	-20.00	1.0	0.9	0.9	0.9	0.1	weak
		4.00	0.7	0.8	0.7	0.7	0.1	weak
		25.00	0.0	0.0	0.0	0.0	0.0	none
		37.00	0.0	0.0	0.0	0.0	0.0	none
		65.00	0.0	0.0	0.0	0.0	0.0	none
	control	4.00	1.0	0.8	0.9	0.9	0.1	weak
t = 2 days	Pyocin PA18	-20.00	1.0	1.0	1.1	1.0	0.1	weak
		4.00	1.0	0.9	1.0	1.0	0.1	weak
		25.00	0.0	0.0	0.0	0.0	0.0	none
		37.00	0.0	0.0	0.0	0.0	0.0	none
		65.00	0.0	0.0	0.0	0.0	0.0	none
	control	4.00	0.9	1.0	1.0	1.0	0.1	weak
t = 5 days	Pyocin PA18	-20.00	1.0	0.8	1.0	0.9	0.1	weak
		4.00	0.0	0.0	0.0	0.0	0.0	weak
		25.00	0.0	0.0	0.0	0.0	0.0	none
		37.00	0.0	0.0	0.0	0.0	0.0	none
		65.00	0.0	0.0	0.0	0.0	0.0	none
	control	4.00	0.0	0.0	0.0	0.0	0.0	none
t = 10 days	Pyocin PA18	-20.00						
		4.00						
		25.00						
		37.00						
		65.00						
	control	4.00						

Table 3C. Pyocin PA31 Stability Data

Pyocin PA31 Stability Data								
		trmt	**rep1**	**rep2**	**rep3**	**mean**	**sd**	**pheno**
t = 0 hr	control	4.00	0.90	0.80	0.90	0.87	0.06	v weak
t = 6 hr	Pyocin PA31	-20.00	1.00	0.90	0.70	0.87	0.15	v weak
		4.00	0.90	0.70	0.80	0.80	0.10	v weak
		25.00	0.90	0.90	0.80	0.87	0.06	v weak
		37.00	0.80	0.90	0.70	0.80	0.10	v weak
		65.00	0.00	0.00	0.00	0.00	0.00	none
	control	4.00	0.90	0.80	0.90	0.87	0.06	v weak
t = 28 hr	Pyocin PA31	-20.00	1.00	0.90	0.80	0.90	0.10	v weak
		4.00	0.70	0.70	0.80	0.73	0.06	v weak
		25.00	1.00	1.00	1.00	1.00	0.00	v weak
		37.00	0.00	0.00	0.00	0.00	0.00	none
		65.00	0.00	0.00	0.00	0.00	0.00	none
	control	4.00	1.00	0.90	0.70	0.87	0.15	v weak
t = 36 hr	Pyocin PA31	-20.00	1.00	1.00	1.00	1.00	0.00	none
		4.00	1.00	0.90	0.90	0.93	0.06	none
		25.00	0.00	0.00	0.00	0.00	0.00	none
		37.00	0.00	0.00	0.00	0.00	0.00	none
		65.00	0.00	0.00	0.00	0.00	0.00	none
	control	4.00	0.90	0.90	0.90	0.90	0.00	none
t = 5 days	Pyocin PA31	-20.00	1.00	1.10	1.00	0.93	0.12	weak
		4.00	0.00	0.00	0.00	0.00	0.00	none
		25.00	0.00	0.00	0.00	0.00	0.00	none
		37.00	0.00	0.00	0.00	0.00	0.00	none
		65.00	0.00	0.00	0.00	0.00	0.00	none
	control	4.00	0.00	0.00	0.00	0.00	0.00	none
t = 10 days	Pyocin PA31	-20.00						
		4.00						
		25.00						
		37.00						
		65.00						
	control	4.00						

Table 3D. Pyocin AP41 Stability Data

Pyocin AP41 Stability Data								
		trmt	rep1	rep2	rep3	mean	sd	pheno
t = 0 hr	control	4.00	1.20	1.20	1.20	1.20	0.00	strong
t = 6 hr	Pyocin AP41	-20.00	1.10	1.00	1.10	1.07	0.06	strong
		4.00	1.20	1.10	1.10	1.13	0.06	strong
		25.00	1.10	1.00	1.10	1.07	0.06	strong
		37.00	1.00	1.10	1.00	1.03	0.06	strong
		65.00	0.00	0.00	0.00	0.00	0.00	none
	control	4.00	0.90	0.80	0.90	0.87	0.06	strong
t = 28 hr	Pyocin AP41	-20.00	1.20	1.10	1.00	1.10	0.10	strong
		4.00	1.00	1.00	1.00	1.00	0.00	strong
		25.00	1.20	1.10	1.10	1.13	0.06	strong
		37.00	1.00	1.10	1.10	1.07	0.06	strong
		65.00	0.00	0.00	0.00	0.00	0.00	none
	control	4.00	1.10	1.10	1.30	1.17	0.12	strong
t = 36 hr	Pyocin AP41	-20.00	1.10	1.20	1.10	1.13	0.06	strong
		4.00	1.10	1.10	1.10	1.10	0.00	strong
		25.00	0.90	1.10	1.00	1.00	0.10	strong
		37.00	1.00	1.00	1.00	1.00	0.00	strong
		65.00	0.00	0.00	0.00	0.00	0.00	none
	control	4.00	1.10	1.20	1.10	1.13	0.06	strong
t = 5 days	Pyocin AP41	-20.00	1.00	1.00	1.00	1.00	0.00	weak
		4.00	0.80	0.90	0.90	0.87	0.06	weak
		25.00	0.00	0.00	0.00	0.00	0.00	none
		37.00	0.00	0.00	0.00	0.00	0.00	none
		65.00	0.00	0.00	0.00	0.00	0.00	none
	control	4.00	0.80	0.90	0.90	0.87	0.06	none
t = 10 days	Pyocin AP41	-20.00						
		4.00						
		25.00						
		37.00						
		65.00						
	control	4.00						

Table 3E. Bacillus FD7g Stability Data

Bacillus FD7g Bacteriocin Stability Data								
		trmt	**rep1**	**rep2**	**rep3**	**mean**	**sd**	**pheno**
t = 0 hr	control	4.00	1.80	1.70	1.50	1.67	0.15	weak
t = 6 hr	Bacillus FD7g	-20.00	1.80	1.80	1.80	1.80	0.00	weak
		4.00	1.80	1.80	1.80	1.80	0.00	weak
		25.00	1.80	1.80	1.80	1.80	0.00	weak
		37.00	1.80	1.80	1.80	1.80	0.00	weak
		65.00	0.00	0.00	0.00	0.00	0.00	none
	control	4.00	0.90	0.80	0.90	0.87	0.06	weak
t = 28 hr	Bacillus FD7g	-20.00	1.60	1.50	1.50	1.53	0.06	none
		4.00	1.10	0.90	1.20	1.07	0.15	none
		25.00	0.00	0.00	0.00	0.00	0.00	none
		37.00	0.00	0.00	0.00	0.00	0.00	none
		65.00	0.00	0.00	0.00	0.00	0.00	none
	control	4.00	1.10	0.90	1.10	1.03	0.12	none
t = 36 hr	Bacillus FD7g	-20.00	1.50	1.20	1.00	1.23	0.25	none
		4.00	1.10	1.30	1.20	1.20	0.10	none
		25.00	0.00	0.00	0.00	0.00	0.00	none
		37.00	0.00	0.00	0.00	0.00	0.00	none
		65.00	0.00	0.00	0.00	0.00	0.00	none
	control	4.00	1.10	1.20	1.20	1.17	0.06	none
t = 5 days	Bacillus FD7g	-20.00	1.00	0.80	1.00	0.93	0.12	weak
		4.00	1.10	1.00	1.00	1.03	0.06	none
		25.00	0.00	0.00	0.00	0.00	0.00	none
		37.00	0.00	0.00	0.00	0.00	0.00	none
		65.00	0.00	0.00	0.00	0.00	0.00	none
	control	4.00	1.00	1.00	1.00	1.00	0.00	none
t = 5 days	Bacillus FD7g	-20.00						
		4.00						
		25.00						
		37.00						
		65.00						
	control	4.00						

Table 3F. Colicin E1 Stability Data (Control)

Colicin E1 Lysate Stability Data									Pure Colicin E1 Freeze/Thaw Stability				
time and temperature													
		trmt	rep1	rep2	rep3	mean	sd	pheno	-20C	rep1	rep2	mean	sd
t = 0 hr	control	4.00	1.3	1.1	1.1	1.2	0.1	strong	t=0	1.50	1.50	1.50	0.00
t = 6 hr	Colicin E1	-20.00	1.2	0.9	1.0	1.0	0.2	weak					
		4.00	1.3	1.1	1.1	1.2	0.1	strong					
		25.00	1.1	0.9	1.0	1.0	0.1	strong					
		37.00	1.0	1.0	0.7	0.9	0.2	strong					
		65.00	0.0	0.0	0.0	0.0	0.0	none					
	control	4.00	1.1	1.0	0.9	1.0	0.1	strong	t=6	1.50	1.40	1.45	0.07
t = 1 day	Colicin E1	-20.00	1.0	1.0	0.9	1.0	0.1	weak					
		4.00	0.8	0.9	0.7	0.8	0.1	strong					
		25.00	0.8	0.8	1.0	0.9	0.1	strong					
		37.00	0.8	0.8	0.8	0.8	0.0	strong					
		65.00	0.0	0.0	0.0	0.0	0.0	none					
	control	4.00	1.0	0.9	0.8	0.9	0.1	strong	t = 1 day	1.40	1.40	1.40	0.00
t = 2 days	Colicin E1	-20.00	0.7	0.9	0.6	0.7	0.2	strong					
		4.00	0.8	0.9	0.9	0.9	0.1	strong					
		25.00	1.1	0.9	1.0	1.0	0.1	strong					
		37.00	1.1	1.0	1.1	1.1	0.1	strong					
		65.00	0.0	0.0	0.0	0.0	0.0	none					
	control	4.00	0.9	0.9	0.6	0.8	0.2	strong	t = 2 days	1.50	1.50	1.50	0.00
t= 5 days	Colicin E1	-20.00	1.0	1.0	0.9	1.0	0.1	strong					
		4.00	1.0	0.9	1.0	1.0	0.1	strong					
		25.00	1.0	1.0	1.0	1.0	0.0	strong					
		37.00	1.0	1.0	0.9	1.0	0.1	strong					
		65.00	0.0	0.0	0.0	0.0	0.0	none					
	control	4.00	1.0	1.0	1.0	1.0	0.0	strong	t = 5 days				
t = 10 days	Colicin E1	-20.00	0.0										
		4.00	0.0										
		25.00	0.0										
		37.00	0.0										
		65.00	0.0										
	control	4.00	0.0						t = 10 days				

Table 4. Bacteriocin Chemical Stability Study

	Solution	Solution	Solution + Water	ColE1 Lysate	ColE1 Pure	Nisin	AP41	FD7b
1	acetone	0	0	1	1	1	1	1
2	chloroform	1	0	1	1	1	1	1
3	ETOH	0	0	1	1	1	1	1
4	palmolive	1	0	0	0	1	0	1
5	NaCl 500 mM	0	0	1	1	1	1	1
6	glycerol	0	0	1	1	1	1	1
7	lubriderm	0	0	1	1	1	1	1
8	bleach	1	1	1	1	weak	1	1
9	HCL	0	0	1	1	1	1	1
10	NaOH	1	1	1	1	1	1	1
11	urea 20 %	0	0	1	1	1	1	1
12	MgCl2 500 mM	0	0	1	1	1	1	1
13	soft soap	0	0	0	1	1	0	1
14	milk	0	0	1	1	1	1	1
15	apple juice	0	0	1	1	1	1	0
16	1X PBS	0	0	1	1	1	1	1
17	CaCl2 100 mM	0	0	1	1	1	1	1
18	EDTA 0.5 M	1	1	1	1	1	1	1
19	Gelatin 2%	0	0	1	1	1	1	1
20	sucrose 50%	0	0	1	1	1	1	0
21	proteinase K	0	0	0	0	1	0	0
22	trypsin	0	0	0	0	1	0	0
23	PEG 20%	0	0	1	1	1	1	1
24	borax 10%	0	0	1	1	1	1	1
25	boil	0	0	0	weak	1	0	1
26	autoclave	0	0	0	0	1	0	0
27	microwave	0	0	0	weak	1	1	1
28	UV	0	0	1	1	1	1	1
29	freeze thaw X1	0	0	1	1	1	1	1
30	freeze thaw X2	0	0	1	1	1	1	1
31	freeze thaw X3	0	0	1	1	1	1	1
32	bacteriocin at 4C	0	0	1	1	1	1	1

1 = lysate active

0= lysate inactive

Chemical itself had antimicrobial activity

Chemical inactivated lysate antimicrobial activity

nisin pure came from powder - solution was 250 mg into 3ml

colicin E1 pure came from the B series bottles (~150 mg/ml)

Year Three

Phase four involves comparing killing breadths and rates of resistance emergence for bacteriocin candidates against mixed species bacterial samples.

Summary: Year three was re-scoped to entail analysis of the genomic sequences of the candidates to identify the bacteriocins causing the killing activity. The genomes of the following candidates are being analyzed for the presence of bacteriocins: PA-15, PA-18, PA-31, FD3b, FD7gb, FD8x, FD10f.